The development, structure and repair of articular cartilage

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Translation from Research to Applications

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ABSTRACT

The article summarizes the collective views expressed at the fourth session of the workshop Tissue Engineering—the Next Generation, which was devoted to the translation of results of tissue engineering research into applications. Ernst Hunziker described the paradigm of a dual translational approach, and argued that tissue engineering should be guided by the dimensions and physiological setting of the bodily compartment to be repaired. Myron Spector discussed collagen-glycosaminoglycan (GAG) scaffolds for musculoskeletal tissue engineering. Jeanette Libera focused on the biological and clinical aspects of cartilage tissue engineering, and described a completely autologous procedure for engineering cartilage using the patient's own chondrocytes and blood serum. Arthur Gertzman reviewed the applications of allograft tissues in orthopedic surgery, and outlined the potential of allograft tissues as models for biological and medical studies. Savio Woo discussed a list of functional tissue engineering approaches designed to restore the biochemical and biomechanical properties of injured ligaments and tendons to be closer to that of the normal tissues. Specific examples of using biological scaffolds that have chemoattractants as well as growth factors with unique contact guidance properties to improve their healing process were shown. Anthony Ratcliffe discussed the translation of the results of research into products that are profitable and meet regulatory requirements. Michael Lysaght challenged the proposition that commercial and clinical failures of early tissue engineering products demonstrate a need for more focus on basic research. Arthur Coury described the evolution of tissue engineering products based on the example of Genzyme, and how various definitions of success and failure can affect perceptions and policies relative to the status and advancement of the field of tissue engineering.

INTRODUCTION

THE TECHNOLOGY OF TISSUE ENGINEERING has been shown to be feasible; some products are already on the market and there is potential for the development of new products with significant clinical impact. Translation of research from the laboratory to the clinic requires animal studies, and many questions remain about the suitable animal models for human conditions, particularly in cases where the main clinical outcome variable is pain relief. Long-term rather than short-term

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investment money, business plans geared to realistic cost/benefit trade-offs, less hype, more sophisticated personnel skilled at product development and manufacturing scale-up are needed to move the field toward the clinic. Along with these, continued progress on the fundamental side is needed to provide support for the translational advancements.

FUNCTIONAL TISSUE ENGINEERING: PARADIGM OF A DUAL TRANSLATIONAL APPROACH

Ernst Hunziker from the University of Bern, Bern, Switzerland, described the concept of functional tissue engineering (FTE) as an applied science, and proposed the paradigm of a dual translational approach. He made the case that practical tissue engineering should be approached with a clear view of the bodily compartment to be repaired. Not only the dimensions of the defect and its etiology, but also the physiological setting and its influences must be known. To gain an insight into the biological factors at play within an implanted construct, the ''raw'' product must be subjected to a short- and midterm troubleshooting investigation *in vivo*. The level of adverse foreign-body reactivity, the ingress of undesired cell populations from neighboring tissue compartments, and the influence of local signaling activity must be evaluated. Ways must be found to deal with these problems along a course that cooperates rather than contends with the desired tissue-compartment-specific repair response. After having made the necessary theoretical adjustments to the engineering principle, the predictors of success identified in the initial *in vivo* experiments can be optimized in a first translational phase *in vitro*. The quality, functionality, and durability of the *in vitro*–optimized tissue constructs can then be evaluated in a second, and final, translation phase *in vivo*, initially in the short- and midterms, and subsequently in the long-term—at 6 days, 6 weeks, and 6 months.

Tissue engineering is an interdisciplinary science. For the promise of success, advice of specialists in all disciplines related to construct development should be sought—from the initial concept, through experimental testing, to potential industrial manufacture for clinical use—so as to anticipate and circumvent as many difficulties as possible before these converge to become a Gordian knot. Tissue engineering is also an applied science, which aims to restore tissue activities that have been undermined by destruction or degeneration, and which either cannot be reestablished by available therapeutic means, or, if they can, depend upon the transplantation of cellular material. To improve our knowledge and understanding of biological systems, we tend to design experiments that will reveal the fundamental processes and the underlying mechanisms. The insight thereby gained is then applied to develop tools that will selectively inhibit or enhance system components.

Successful tissue engineering depends on a thorough knowledge and understanding of the biological system in hand. But instead of experimentally stripping the system to reveal its components, the tissue engineering approach takes these components, or suitable substitutes, with a view of reestablishing the system structurally and functionally. Inevitably, therefore, most tissue engineering approaches are initially of an essentially empirical nature, the concept being tested first *in vitro* and then *in vivo*. However, many strategies that appear to be promising *in vitro* yield disappointing results when translated to living organisms, thereby bringing home to us the complexity of the natural system and our incomplete grasp of its intricacies. The impact of one biological phenomenon in particular, namely, immunoreactivity and tissue rejection, cannot be fully appreciated *in vitro*. Engineered tissue will be treated by the body as a foreign object. Hence, it is not surprising that many new concepts fail due to inadequate testing *in vivo*.

Identification of the medical problem and of the therapeutic needs

The medical problem to be solved must be defined not only symptomatically but also structurally. By way of illustration, Dr. Hunziker will refer to his own studies on the repair of structural lesions in the articular cartilage layer of synovial joints.[1,2] Such lesions can arise either traumatically, or pathologically during the course of osteoarthritis. They may be confined to the avascular layer of articular cartilage (partial-thickness defects) or may penetrate the sanguinous subchondral bony compartment (full-thickness defects). The biology of these two types of defects differs greatly, and must be accounted for in the choice of treatment. Partial-thickness defects manifest no spontaneous repair activity, whereas full-thickness ones do. Moreover, when the subchondral bone is involved, two different types of tissue need to be repaired, one of which is vascularized and the other is not, and this distinction must be preserved if joint function is to be fully restored. Hence, it does not suffice merely to identify the presence of a ''joint'' lesion; the extent of the structural damage incurred must be established.

Tissue engineering systems

Once the nature of the medical problem to be tackled has been established, the type of system that we wish to elaborate must be selected from the three existing alternatives: an acellular approach, a cell-based approach, and a combined approach. Acellular approaches aim to facilitate the repair activity of native cell populations. They may consist just of a nude matrix, or of a matrix containing biological signaling agents that will recruit and trigger the differentiation of local stem cell populations, thereby resulting in the formation of a functionally competent type of repair tissue. Cell-based approaches can involve adult stem cells, embryonic stem cells, or differentiated cells. If immature cells are used, these

may either be left to differentiate *in vivo* or be induced to do so *in vitro*, prior to implantation. The cells can be applied either alone or in conjunction with a matrix. The combined approach is the most popular of the three alternatives. In such systems, cell populations are trapped within a matrix that is functionalized with a biological signaling agent. Likewise with this approach, cell maturation can be effected either prior to or after implantation.

At this conceptual stage, investigators should also decide on the intended mode of application of their engineered product—whether it is to be introduced surgically or arthroscopically, or as a solid entity or in an injectable form—whether one or several interventions will be necessary, and if more than one is required, whether these are likely to be acceptable and well tolerated.

Clinical product considerations

An awareness of the likely costs of manufacture of a construct for clinical use is also essential nowadays. If a construct is too costly to produce, it is unlikely to have any market value. Investigators with a purely scientific background are not accustomed to think along these lines, which is why tissue engineering should, ideally, be an interdisciplinary enterprise. The advice of persons with industrial and marketing experience should be sought early, at the conceptual stage of the project. Clinical input relating to the importance of the medical problem to be tackled and a practicable and convenient mode of applying the engineered product is also essential. Not only clinical practitioners but also public health authorities should be consulted, in order to assess the social impact of the medical problem and to gain some idea of the size of the patient population that is likely to benefit from the envisioned tissue engineering strategy. Further, it should be borne in mind that the end product destined for medical use will be subject to the stringent evaluation process that is imposed on all potential therapeutic tools prior to their approval. To facilitate and expedite this screening at a later stage, all experiments conducted should be meticulously documented. If due and timely consideration is given to these various issues, the tissue engineering project will be highly focused, with clear aims in view, and will proceed in a direction that is most likely to yield a simple, effective, and marketable product.

Initial evaluation of an engineered construct in vivo

In principle, tissue engineering approaches deal with the control of wound-healing processes and aim to boost or redirect the body's own capacity for repair. If a therapeutic intervention is to be efficient, then we must take into account the natural healing responses occurring at the destined site of implantation, and thoroughly understand the biological conditions operating physiologically and in a pathological state. These issues, and the relevant factors at play, can be assessed only when the engineered construct is implanted in living animals.

When a construct is implanted, the initial local reaction will be such as that occurs following any type of tissue injury or trauma that involves hemorrhaging, the formation of a hematoma, and blood clotting (Table 1). Blood-borne components released from a lesioned vessel are recognized as foreign material and they trigger an inflammatory response, which is mediated by macrophages (and, to a lesser extent, by foreign-body giant cells). This reaction leads to the resorption of the fibrinous clot and its substitution, first by granulation tissue and then by a primitive type of vascularized scar tissue laid down by the invading population of fibroblasts. Although this repair tissue is unspecific and does not correspond in type to the compartment within which it is deposited, it is nevertheless biocompatible and thus persists (Table 1). An implanted construct will trigger a similar inflammatory reaction, which will lead first to its resorption by foreign-body giant cells and macrophages, and then, following vascular invasion, to its replacement with unspecific scar tissue (Table 2).

Tissue biocompatibility is a tissue-compartment-specific phenomenon. The only type of tissue that is universally biocompatible with all five mammalian tissue types is the unspecific scar tissue. Hence, the art of tissue engineering lies in our ability to control and to redirect the remodeling process that accompanies the resorption of a construct, in such a manner as to lead to its replacement, not by an unspecific type of scar tissue but by a compartment-specific type of functional parenchymal tissue.

A true conception of the type of tissue that needs to be engineered can be gained only if the microenvironment of the destined implantation site is thoroughly understood in terms of its biology, mechanical characteristics, and the immunological backdrop. The topographical location of the implantation site as well as the dimensions of the lesion must

TABLE 1. LOCAL TISSUE RESPONSE TO INJURY AND TRAUMA

Hemorrhaging from lesioned vessels and blood clotting
↓
Blood-bome physiological components, leaking from the usually closed sanguinous compartment, are recognized as foreign material by the surrounding tissue, since they are not tissue-specific.
↓
Inflammatory response mediated by macrophages (and more rarely by foreign-body giant cells)
↓
Resorption of blood clot
↓
Ingrowth of blood vessels and fibroblasts, and the formation of granulation tissue
↓
Deposition of an unspecific but biocompatible type of repair (scar) tissue by fibroblasts

Table 2. Local Tissue Response to an Implanted Construct

Inflammation
↓
Resorption
↓
Substitution
↓
Vascular invasion and scar-tissue formation

be defined, since the volume of a construct will have an important bearing on its chances of survival in the long run. Hence, in the initial animal studies, the influence of a lesion's dimensions on repair must be analyzed. The assessment of newly formed tissue should also include an evaluation of its integration with the surroundings, which is essential for its structural and functional competence, and of its connectivity with the existing blood, lymphatic, and nervous systems (if relevant). Investigators should also ascertain whether local adult stem cell pools are available for recruitment, and whether these can be efficiently stimulated to migrate and proliferate, and to repair small as well as large structural defects (for examples, see Hunziker *et al.*[1,2,3,4]).

A problem that is frequently overlooked by investigators is the local presence of unwanted cells with a high proliferative capacity, such as fibroblasts, which could invade the construct and remodel it into unspecific scar tissue. The migration of such cells into the defect site can be prevented by applying the principles of structural or functional guided tissue regeneration.[3,4] A classical example of this problem is the repair of atrophic bony defects in the mandible or maxilla. In this case, structural barriers are introduced to prevent epithelial cells and fibroblasts from invading the implanted construct.[5,6]

The importance of the mechanical microenvironment is frequently underestimated or even ignored in situations that do not involve components of the musculoskeletal apparatus. The cardiovascular system, for example, has a very important mechanical function of pumping blood around the entire body. The mechanical forces operating within the microenvironment of the lesion during tissue remodeling will have an important bearing on repair.[7] If these mechanical considerations are neglected, the remodeling process is unlikely to yield a tissue with the native structural characteristics, which depend partially on the mechanical stress fields operating during the terminal differentiation of cells in the repair tissue, and which are required for the mechanical competence of the tissue.

One of the most important issues to be tackled is the adverse reactivity of immunocomponent cells, such as macrophages, foreign-body giant cells, and lymphocytes. Success in tissue engineering depends greatly on our ability to control the foreign-body reaction and to steer it along a course that will cooperate rather than contend with the directed remodeling process. This point is best illustrated by an example drawn from Dr. Hunziker's studies on bone repair (implant osseointegration). Titanium alloy discs coated biomimetically with a functionalized layer of calcium phosphate were implanted at an ectopic (subcutaneous) site in rats.[8] During the early postoperative phase, foreign-body giant cells were observed to invade the implantation site and to actively degrade the calcium phosphate coatings. The osteogenic agent incorporated into the inorganic lattice work was consequently freed by the activity of these foreign-body cells, and thus made available for the recruitment of osteoprogenitor cells and their transformation into osteoblasts, which then dominated the scene.[8]

It is also worthwhile to test for the species-specificity of the engineered construct. If this limitation exists, then the concept must be adapted to render it universally applicable. Hence, the construct should be evaluated in several animal species at an early stage.

To recapitulate, these initial short- and midterm experiments with living animals should aim at identifying all the variables operating at the prospective implantation site. In the light of the information thereby gained, it will then be necessary to redefine and fine-tune the initial concept, and to abandon the empirical approach in favor of a more rational one. In Dr. Hunziker's studies relating to the repair of partial-thickness defects in articular cartilage, exploratory animal experiments of the kind described above revealed many of the local biological factors that can undermine the healing response[1] (Fig. 1). Having identified the intrinsic stumbling blocks, it was possible to overcome these by an informed, rational, and systematic approach.[2] After having made the necessary adjustments to the engineering principle, investigators can then proceed to the next experimental phase or the first translation phase, the purpose of which is to optimize *in vitro* the predictors of success identified in the initial *in vivo* experiments.

First translation

This experimental fine-tuning phase is conducted *in vitro*, rather than *in vivo*, mainly because the expense of exhaustive testing in living animals would be too great. In this first translation phase, the predictors of success identified in the initial animal experiments must be systematically investigated to optimize the repair results achieved with the construct after implantation. First and foremost, the *in vitro* system should simulate as closely as possible the local "bioreactor" conditions operating *in vivo*. For example, the dimensions of the construct should correspond to those of the lesion, and the mechanical microenvironment, as well as the immunological scenery, pertaining locally *in vivo* should be imitated. The quality of the repair tissue can be optimized by testing the effects of various signaling agents on cell differentiation and the production of a tissue-specific extracellular matrix (ECM). Aspects relating to the nutritional limitations of the construct and to the permeation of signaling agents can also be investigated in relation to its

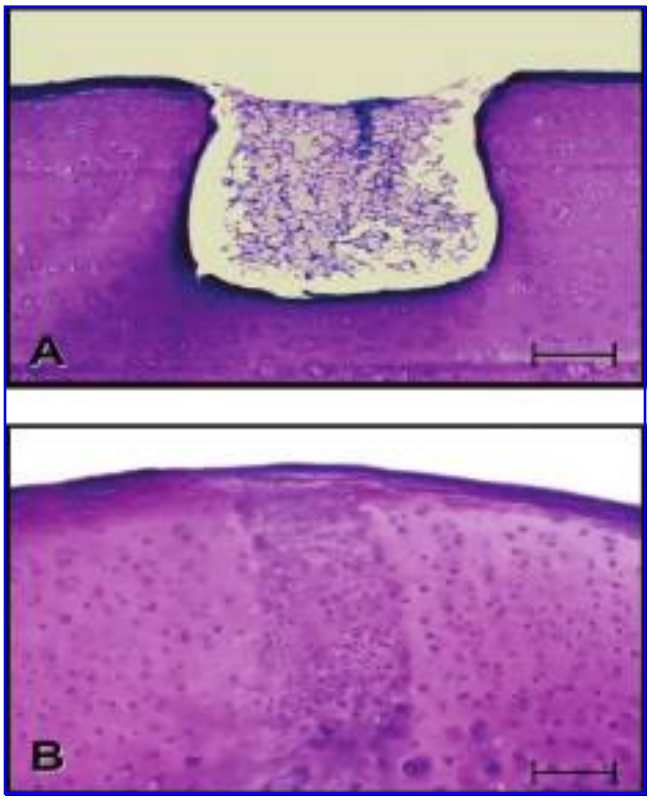

FIG. 1. Partial-thickness articular cartilage defects do not heal spontaneously. Further, they cannot be induced to repair simply by the local application of a chemotactic/mitogenic growth factor; such an agent triggers the migration of mesenchymal stem cells from the synovium into the lesion, along the floor of which they form mono- or bilayers. Hence, the proliferative response of these cells is abortive. For these cells to fill the defect volume, this space must be defined by a matrix containing the chemotactic/mitogenic agent (**A**). Under these conditions, the cells lay down a primitive type of repair tissue, which does not transform into cartilage. To effect chondrogenesis and the formation of cartilage-like tissue (**B**), a chondrogenic differentiation factor must be introduced into the matrix in a liposome-encapsulated form (for delayed release at a timely juncture), together with the free chemotactic/mitogenic agent. Bars in (**A**) and (**B**) = 125 μm. Color images available online at www.liebertpub.com/ten.

size. And, to a limited extent, the problems of tissue integration can be addressed *in vitro*. The *in vitro* process of optimization and refinement helps to perfect the product, thereby lessening the expense and improving the efficiency of the second, and final, phase of testing *in vivo*.

Second translation: In vivo *adaptation of the system, and optimization of its efficacy, safety, and long-term functionality*

In this second, and final, *in vivo* phase, the principle of the system is put to the test. In order to acquire a full picture of what is taking place in the living organism, the fate of the implanted construct should be monitored at three time points, which reflect crucial events in the usual course of repair. As a rule of thumb, suitable sampling intervals would be 6 days, 6 weeks, and 6 months.

During the initial 6 to 12 postoperative days, the magnitude of the inflammatory response, the level of immunological activity, and the extent of implant resorption will be revealed. During the intermediate 6- to 10-week interval, it will be possible to ascertain whether a structurally and functionally relevant tissue has been formed as a result of the remodeling process. To establish this fact, the tissue must be thoroughly characterized. If the evaluation yields promising results, then the investigation can proceed to the 6-month, or, preferably, the 1-year stage, at which juncture the long-term structural and functional competence of the remodeled tissue should be reevaluated (Table 3). Obviously, the suggested time points should be taken *cum grano salis*, and will need to be adapted to the individual case.

Probing during the first week of implantation will yield information respecting the severity of the native tissue's response to the construct, and will indicate whether the engineering principle is operating successfully to redirect the "adverse" reactivity in a "positive" direction, namely, toward the formation of a site-specific type of repair tissue. At this stage, and together with the surgeon's input respecting the ease and practicability of the implant-application mode, it will be possible to assess whether the surgical technique is an appropriate one to elicit the desired course of events. The quality of implant integration with native tissue should also be evaluated, since a good cohesion result at this stage will bode well (and vice versa) for an optimal bonding of the remodeled construct with the defect borders. The suitability of the method chosen for implant fixation will also be revealed at this early time point. Data gleaned from the first *in vivo* phase of the project will help complete the pool of information garnered at the short-term juncture.

An analysis performed at the midterm, 6- to 10-week juncture will reveal whether the construct has been remodeled according to our hypothetical expectations into a structurally and functionally relevant type of repair tissue. The 6-month or 1-year evaluation will indicate whether the

TABLE 3. TIME POINTS FOR MONITORING IN THE SECOND AND FINAL *IN VIVO* PHASE OF THE INVESTIGATION: 6 DAYS, 6 WEEKS, AND 6 MONTHS

Short term, 6-day, juncture:	Evaluation of the inflammatory response, of immunoreactivity, and of implant resorption.
Mid-term, 6-week, juncture:	Has a structurally and functionally relevant tissue been remodeled from the implanted construct?
Long-term, 6-month, juncture:	Has the remodeled tissue persisted in a structurally and functionally competent form?

structural and functional competence of this tissue is likely to persist in the long run.

Animal experiments must be thoroughly thought out with respect to the variables at play in the system. It is indispensable that the appropriate negative and positive control groups are established, and that each of the variables is tested individually. Further, each group should be established in at least two different animal types, in order to check out the species-specificity of the construct. The more complex the system, the more complex will be the experimental design (and the lesser the chance of yielding a marketable product). The tissue characterization to be undertaken at the mid- and long-term junctures should include a rigorous quantitative evaluation of well-defined structural parameters, quantitative immunohistochemistry, and the biochemical analysis of appropriate tissue-specific markers (Table 4).

It cannot be too emphatically stressed that the histological analysis of repair-tissue quality should not be based on subjective scoring schemes, which are all but useless. Well-defined and biologically meaningful structural parameters, which can be readily quantified, are much more powerful. The morphometric analysis of these structural parameters must be based on an appropriate and well-defined tissue-sampling strategy.[9]

The functional characterization will of course depend upon the tissue or organ under investigation. For example, in the case of articular cartilage the mechanical properties of the tissue should be quantified, whereas in the case of the liver the biochemical and secretory competence should be ascertained.

If the midterm analysis yields promising results, then the long-term experiments can be executed. The tissue should not be analyzed until at least 6 months have elapsed from the time of surgery, and preferably not before 1 year. If possible, the implantation site should be periodically surveyed during the postoperative course using *in vivo* monitoring techniques, such as X-radiography or MRI. In parallel with the characterization of the repair tissue, adjacent tissues should be subjected to a toxicological analysis. For example, in articular-cartilage-repair studies, not only normal articular cartilage in the defect vicinity, but also vicinal subchondral bone, synovial tissue, synovial fluid, joint ligaments, and joint-associated lymph nodes, as well as regional lymph nodes and serum, should be sampled and analyzed for adverse or even deleterious changes. Toxicological evaluation is important in assessing the physiological impact of the engineered construct, and will be required when the product is submitted for approval by federal authorities.

Table 4. Characterization of the Repair Tissue

Structural organization:	Histological and ultrastructural analysis; immunohistochemistry.
Qualification (morphometric analysis) of the repair-tissue result:	Choice of biologically meaningful and well-defined structural parameters; instigation of well-defined sampling strategy; avoidance of subjective scoring schemes.
Functional analysis:	Assessment of mechanical or biochemical competence.
Durability:	Persistence of tissue-specific characteristics at the long-term juncture.
Species-specificity:	Evaluation in several different animal species.

The ultimate aim of tissue engineering is to develop methodologies whereby structural and functional bodily defects can be healed. Tissues and organs that do not heal spontaneously are induced to repair by attempting to overcome the biological limitations that undermine this process in nature. At the stage of its conception, a tissue engineering principle must be screened to ascertain the size of the patient population that will be targeted, its socioeconomic impact, the feasibility and likely cost of its manufacture, and its surgical practicability. Hence, tissue engineering is necessarily an interdisciplinary enterprise. There exists no magical formula for the engineering of a product that will be universally applicable to all bodily tissues. An engineered construct must be tailor-made on a tissue-specific basis. Ernst Hunziker concluded that in order to yield a product that will be beneficial to the targeted patient population, a tissue engineering principle should be experimentally developed along systematic and, whenever possible, rational lines, and in the light of a thorough understanding of the biological system in hand.

COLLAGEN-GAG SCAFFOLDS FOR MUSCULOSKELETAL TISSUE ENGINEERING

Myron Spector from Harvard Medical School, Boston, MA, discussed the challenges of developing biomaterial scaffolds for tissue engineering and regenerative medicine. The growth of cells in three-dimensional scaffolds continues to provide unique opportunities to observe selected cell behavior as well as to inform changes in scaffold composition and structure that may improve clinical performance. Investigations of cell-scaffold interactions *in vitro* thus continue to offer the opportunity for discoveries of cell biology. In this regard, tissue engineering promises to provide critical new knowledge that will deepen our understanding of the cell phenotypes and enable meaningful advances in tissue engineering and regenerative medicine. These endeavors are notable particularly because there is a growing consensus that the challenges of developing biomaterial scaffolds for tissue engineering and regenerative medicine exceed the challenges that were faced in the cell biological work that led to the phenotypic proliferation of cells *in vitro*, and in genetic engineering that has led to the production of growth factors and cloning of their genes, the other pillars of tissue engineering and regenerative medicine.

Tissue engineering and regenerative medicine can now be pursued because of recent advances in enabling technologies related to the tissue engineering triad of cells, matrices, and regulators. One of the more important technological advances enabling tissue engineering relates to the means of production of the porous, absorbable scaffolds that are required to contain the cells *in vitro* and/or *in vivo*. Control of the pore characteristics, including pore volume fraction, pore diameter, and pore orientation, as well as the chemical composition of the matrix, has played a critical role in the advance of tissue engineering. In order to further advance scaffold-based tissue engineering and regenerative medicine, and facilitate translation of research to clinical implementation, numerous questions will have to be answered. The following provides a framework for posing such questions.

Scaffolds for tissue engineering and regenerative medicine

Scaffolds for engineering bone and soft tissues have been synthesized from an array of synthetic and natural calcium phosphates and myriad synthetic (e.g., polylactic acid [PLA] and polyglycolic acid [PGA]) and natural (e.g., collagen and fibrin) polymers. The underlying concepts guiding the development of scaffolds are predicated on the selected biomaterial or on the method of production of the scaffold. Examples of biomaterials-based approaches include the use of (i) biomaterials that have been frequently employed in other implant applications (e.g., PLA-PGA);[10] (ii) treated natural ECM materials (e.g., inorganic bone);[11] (iii) biomimetics and analogs of ECM (e.g., collagen-GAG[12] and collagen-hydroxyapatite scaffolds);[13] (iv) biopolymers for nanoscale matrix (e.g., self-assembling peptides);[14] and (v) new types of biomaterials designed specifically for tissue engineering scaffolds. Alternatively, the driving force for the design of scaffolds may be the precision (computer) multiscale control of material, architecture, and cells; solid free-form fabrication technologies. This has become possible with the introduction of a wide array of solid free-form fabrication techniques and apparatus.[15] While it is likely that several scaffolds will be suitable for certain clinical applications, it is becoming increasingly important to determine the unique features of certain biomaterials and the methods of production that best favor clinical outcome. Do certain biomaterials offer a greater number of ligands for adhesion proteins and thus offer an advantage of cell attachment or otherwise regulate cell function? Will vascular networks be able to be introduced into large constructs produced by free-form fabrication methods and thus advance organ printing? What is the effect of the mechanical behavior, and change of such behavior with degradation of the scaffold, on tissue formation *in vitro* and *in vivo*?

There are many roles that a scaffold can play in the tissue regeneration process. The potential role of the scaffold as a delivery vehicle for exogenous cells has become increasingly important in a wide variety of tissues and organs in the light of recent advances in the investigation of cell therapy for local repair. Injection of exogenous cells, expanded in number in monolayer culture, is being studied for the treatment of defects and degenerative conditions in many tissues, examples being (i) chondrocytes for the repair of defects in articular cartilage on the surface of joints,[16] (ii) intervertebral disc cells for herniated disc,[17] (iii) stem cells into spinal cord lesions,[18] (iv) myoblasts and stem cells for myocardial infarction,[19] and (v) cells into the retina.[20]

An alternative to the injection of cells is implantation of a cell-seeded scaffold. As noted above, the large surface area of porous scaffolds allows for the delivery of an exceedingly large number of attached cells, and facilitates the retention of the cells at the implant site. Questions related to the use of scaffolds for these applications include the specific pore structures that will be necessary to accommodate cell function. Moreover, that regulatory molecules may be required to stimulate certain cell functions raises questions about the best way to have the scaffold deliver recombinant proteins or their genes.

Investigations of cell-scaffold interactions in vitro

Investigations of cell-scaffold interactions *in vitro* can inform the rational formulation of scaffold composition and structure for improved performance in tissue engineering and regenerative medicine applications. These investigations of cells in three-dimensional scaffolds that may mimic certain aspects of the natural ECM *in vivo* can also provide insights into, and discoveries of, cell biology. For example, in the course of investigations of the behavior of articular chondrocytes in collagen-GAG scaffolds, it was observed that the disc-shaped scaffolds were decreasing in size.[21] Subsequent histological studies demonstrated a reduction in the pore diameter of the matrices and suggested a cell-mediated process. This led to the finding that chondrocytes were expressing the muscle actin isoform, α-smooth muscle actin (SMA). Following were findings that adult canine and human articular chondrocytes, and many other connective tissue cells and their mesenchymal stem cell progenitors express SMA and can contract.[22] These findings have suggested roles for the contractile behavior of connective tissue cells in the control of the architecture of the ECM and in the response of the tissue to injury. Many new insights into cell biology may be gained from the continued study of the behavior of cells in three-dimensional scaffolds.

Chondrocyte-seeded collagen-GAG scaffolds for cartilage repair

Translating knowledge about the cell-scaffold interactions acquired from research *in vitro* to clinical applications has led to investigations of chondrocyte-seeded scaffolds for cartilage repair procedures. Studies demonstrating the potential benefit of injection of culture-expanded chondrocytes

for cartilage repair date back to rabbit studies first performed in the mid-1980s.[23,24] While subsequent experiments of autologous chondrocyte implantation (ACI) in a canine model[25,26] were less promising, the procedure has been introduced into widespread clinical use[16] with promising symptomatic relief in many patients.[16,27]

Current efforts in many laboratories around the world are being directed toward determining whether the results of ACI can be improved if the cells are implanted as a cell-seeded scaffold rather than delivered by injection. As noted earlier, one design approach has been to employ scaffolds that can serve as analogs of the ECM of the tissue to be engineered.[12] This concept recognizes that the molecular composition and architecture of the ECM display chemical and mechanical properties required by the parenchymal cells and the physiological demands of the tissue. For a wide array of soft-tissue applications collagen-based biomaterials have been employed,[12] and for articular cartilage, type II collagen—the principal constituent of the tissue—has been commended by prior studies.

One recent series of studies compared the reparative tissue in chondral defects in adult dogs implanted with cultured autologous chondrocytes (CACs) alone, that is, ACI[26] and CAC-seeded type II collagen-GAG scaffolds cultured for 24 hours[28] and 4 weeks[29] prior to implantation. The cell-seeded scaffolds yielded a greater amount of reparative tissue than the sites implanted with the CACs alone. The cell-seeded scaffolds cultured for 24 hours induced more reparative tissue formation than the injection of cells alone, but this tissue was made up of fibrocartilage and fibrous tissue with virtually no hyaline cartilage. The question remains as to the relative importance of amount versus composition of the reparative tissue with respect to providing symptomatic relief for individuals with focal cartilage defects. Related to this point is the fact that the hyaline cartilage found at sites treated by CACs alone and in the collagen scaffolds did not display the architecture of articular cartilage.

Of note was that the greatest amount of reparative tissue was induced in the CAC-seeded scaffold cultured for 4 weeks prior to implantation, and that this group demonstrated the same amount of hyaline and articular cartilage as defects implanted with the cells alone.[29] How developed should the cartilaginous construct be before it is implanted? While these studies demonstrate the promise of implementing scaffolds for cartilage repair, there are potential problems and significant expense associated with the culturing a cell-seeded scaffold for 4 weeks prior to implantation. This focuses attention on the implementation of growth factors to accelerate cell proliferation and matrix synthesis in the scaffolds prior to implantation.[30]

Myron Spector concluded that these and other animal studies underscore the importance of *in vivo* investigation. Translation of research from the laboratory to the clinic will understandably require animal studies. A host of questions revolve around the most suitable animal models for human conditions. While certain tissue engineered constructs may not yet meet the initial expectations for the reproduction of the composition and structure of the target tissues, even in animal models, several should be advanced to human trial, appreciating the inadequacy of any animal experiment to fully model the human condition. This is especially true of problems with the main clinical outcome variable of pain relief.

CARTILAGE ENGINEERING—BIOLOGICAL AND CLINICAL ASPECTS

Jeanette Libera from co.don, a company based in Berlin, Germany, focused on tissue engineering of completely autologous cartilage based on the patient's own chondrocytes and blood serum. She argued that the cell-based therapies already available on the market provide insight into the requirements of therapy development, such as the definition of clinical strategy, *in vitro* engineering and functional tests, and animal and clinical studies. Already during formulation of repair strategies a balance between medical (e.g., availability of cell source, pathology), biological (e.g., regenerative capability of selected cell, scaffold resorption rate), and technical (e.g., intraoperative, technical possibilities for transplantation) practicability and potential has to be found. The long-standing experience with autologous cell-based repair demonstrated that chondrocytes have high potential for tissue engineering even without the use of scaffolds. Stem cells may also provide a valuable alternative for specific indications, for example, when no tissue biopsy can be taken. However, for safe application, stem cells need to be characterized for their carcinogenic risk. Regardless of the cell source and of the use of scaffolds, commercially successful strategies are likely those that are simple with regard to biopsy, manufacturing technique and time, culture additives, characterization of the transplant, and operative techniques.

Chondrogenesis and growth factors

It is well established that chondrogenesis is regulated by growth factors.[31–33] Stimulation of tissue regeneration by application of a single growth factor was already done clinically for bone repair.[34] However, in related studies, counter effects were observed that might be related to the undefined dose of the used growth factor.[35] Therefore, also for cartilage, future research should focus on the use of growth factor mixtures.[36] An important milestone will be the understanding of the *in vivo* differentiation-dependent expression of growth factors and of their receptors. This will provide the basis to selectively influence cell differentiation by regulating expression of growth factors and receptors and/or by adding respective factors. In principle, the latter strategy is more appealing circumventing any problem

associated with transfection of cells for a differentiation-dependent expression of growth factors.

Chondrocyte spheroid culture

Remarkably, differentiation-dependent expression of several growth factors has been observed in the autologous three-dimensional chondrocyte spheroid culture system.[37] This again underlines the high potential of isolated and cultured human chondrocytes to regulate their chondrogenesis. The growth factors released during spheroid remodeling, aggregation, and integration into native cartilage could explain the observed stimulation of chondrocyte differentiation and matrix maturation during spheroid remodeling.[37] Spheroid adhesion and subsequent remodeling are likely the crucial steps in structural and functional integration of engineered constructs into native cartilage.

Integration with host tissues. Structural and functional integration with host tissues is essential for long-term functionality of implants.[38,39] For quantitative analysis of integration of tissue engineered constructs, there are already available *in vitro* models.[40] For implant integration, which is a cell-regulated process, ultrastructural integration of secreted matrix components is necessary.[41–43] Further analysis of adhesion processes and mediators will be necessary for the identification of therapeutic strategies to enhance or secure integration of tissue engineered constructs.[32,44–47] Improved arthroscopic and imaging techniques should permit characterization of tissue integration and maturation during postoperative follow-up. Biomarker imaging technologies, and arthroscopic and mechanical test systems are among the technologies that would support and advance functional characterization of tissue regeneration.

Animal models

To retain first *in vivo* data on safety and efficacy of cell-based therapies, animal models were used. These models were selected to mimic the human situation, for example, with regard to mechanical loading and self-repair processes. Remarkably, *in vitro* studies have shown clearly that species-dependent chondrocyte characteristics have to be taken into account.[48] Culturing articular chondrocytes of the human, sheep, horse, dog, cow, and mini-pig species, we observed differences in monolayer morphology, proliferation, and migration as well as a different potential to form an *in vitro* autologous tissue construct. Compared to human chondrocytes, we found for mini-pig chondrocytes a similar cell differentiation and matrix formation after transferring chondrocytes into the spheroid culture system. Based on this observation, we used this model to analyze the capacity of chondrocyte spheroids to heal cartilage defects.

Jeanette Libera concluded that tissue engineering will certainly require new animal models for developing novel technologies to treat specific indications, for example, large animal model of osteoarthritis without destabilization of joints by meniscectomy or anterior cruciate ligament (ACL) transection.

ALLOGRAFT TISSUES FOR ORTHOPEDIC APPLICATIONS

Arthur Gertzman from Musculoskeletal Transplant Foundation (MTF) discussed successful applications of allograft tissues in orthopedic surgery and outlined the potential of allograft tissues as models for elucidating cell-matrix interactions in orthopedic applications. He reminded that tissue engineering requires three key components, all of which are necessary for success. First, cells are required to provide the genetic instructions and energy system to create the needed new cells. Second, growth factor and cytokines mediate the cell activities. Third, a three-dimensional matrix is required to provide a scaffold upon which the cells and cytokines can act. The need for the three-dimensional nature of the required structure should be self-evident, as all natural biological structures are three-dimensional. Much of historical research has been in a two-dimensional mode of monolayers, a ''flat biology'' approach that may not be predictive of the natural, biological milieu.

Human allograft tissues

Human allograft tissues are widely used in orthopedic surgical procedures in the United States. Allografts are recovered and processed with conventional metallic or polymeric medical device implants, or safer ones, under strict and well-developed protocols that have proven to be as safe as surgery. Both the US Food and Drug Administration (FDA) and the American Association of Tissue Banks have published and enforced extensive rules related to donor selection, processing, storage, and transport. Tissues from complying tissue banks have an unparalleled record of patient safety and successful outcome over the past two decades. Allograft tissues are bone or soft tissues removed from one human and transplanted to another for repair of desired or traumatized tissues.

Load-bearing tissues. Cortical bone is a dense tissue with a specific microstructure based on the osteon and interconnecting channels: Haversian, Volkmann, and cell-sized canaliculi (Fig. 2A). Cancellous bone is open and ''webby,'' with interconnecting trabeculae enclosed in a thin cortical shell. The condyles of long bone and such special bones as the patella are cancellous in nature (Fig. 2B). As determined empirically, allograft bone integrates by a mechanism of ''creeping substitution.''[49] Cancellous bone, with its open structure that is osteoconductive and somewhat osteoinductive, will biointegrate into a host within a few months. Cortical bone, being of higher density, will biointegrate more slowly; a significant portion of the originally implanted

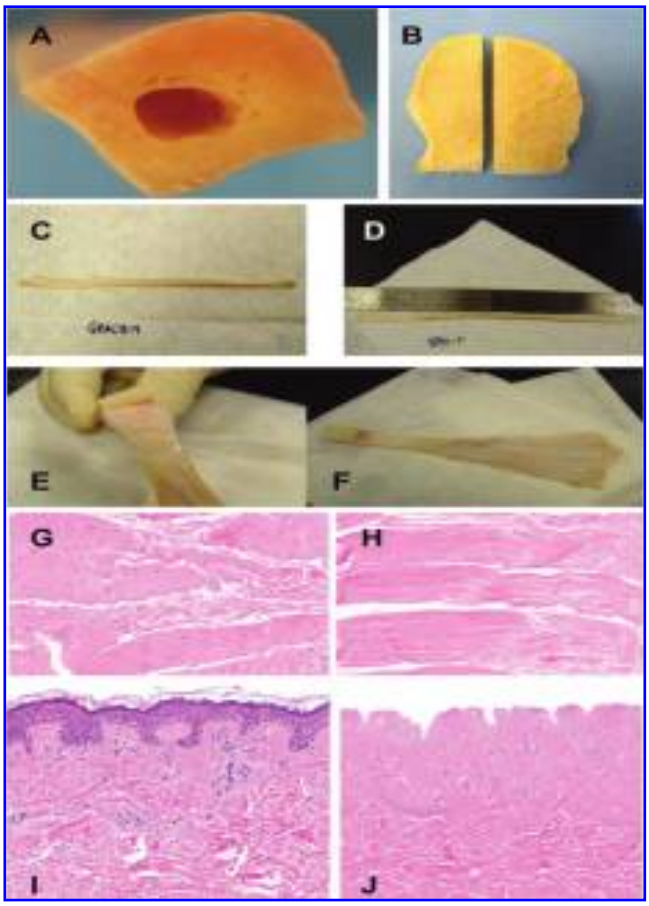

FIG. 2. Human tissue allografts. (**A**) Cortical bone. (**B**) Cancellous bone. (**C**) Gracilus and (**D**) Semi-tendinosus for anterior cruciate ligament (ACL) repair. (**E, F**) Achilles tendon. (**G, H**) Fascia before and after decellularization. (**I, J**) Dermis before and after decellularization. Color images available online at www.liebertpub.com/ten.

cortical bone may be present several years after surgical implantation. Cortical bone has had extensive application in spinal fusion. "Rings" from long bones have been used in anterior and posterior lumbar spinal fusion. The cortical bone has excellent compressive strength. If processed aseptically and not subjected to energy-based sterilization such as gamma irradiation, cortical bone has compressive strength exceeding 25 kN. The use of cortical bone as a load-bearing spacer allows the adjacent vertebrae to fuse under the influence of osteoinductive substances placed adjacent to or within the implant.

A recent design combines the load-bearing properties of cortical bone with the osteoconductive and osteoinductive characteristics of cancellous bone. The design places the load-bearing cortical component in the anterior aspect of the implant, while the cancellous component is placed posteriorally. The fusion occurs first in the posterior zone through the creeping substitution mechanism. This design has been used for 2 years in cervical spinal fusion. Another modification has been devised to create some degree of surface osteoinduction by partially demineralizing a 50 μm thick zone on the cortical surface.

Soft tissues—tendons and ligaments. Several human allograft soft tissues have been adopted for surgical repair, particularly in sports medicine. Several long tendons of the legs, knee, and feet are widely used for repair of the ACL of the knee. The gracilus and semi-tendinosus ligaments (Fig. 2C, D) are used to replace torn ACL. They may be doubled or quadrupled in thickness to provide greater load resistance. Fixation in the tibial and femoral tunnel is accomplished with fixation screws, "buttons," or staples.[50] Similarly, the Achilles tendon (Fig. 2E) with attached talus bone is a popular choice for ACL repair because of its high strength, both in the tendon portion and at the bony insertion (Fig. 2F). The most widely used allograft in sports medicine is the bone-patellar tendon-bone (BPTB). It is recovered from the donor tissue by excising a suitable bone block with its tendon insertion from the tibial condyle (cancellous). The intact contiguous patella and patellar tendon (PT) are also collected, resulting in the BPTB. It is easy to insert and fix into a prepared tunnel with metallic or polymeric fixation screws. The bone blocks are primarily cancellous bone and rapidly incorporate. The tendon portion is very strong and also integrates by serving as a scaffold, which is slowly remodeled by the host tissue. The clinical result restores functionally useful strength over a period of 12–24 months.

Soft tissue—fascia. Fascial tissue is usually recovered from the fascia lata, a primarily collagenous tissue. Commercial processes have been developed to decellularize the tissue in a manner that retains the gross morphology and precludes loss of the tensile properties of the tissue. Decellularization removes cells and collagen and the tissue is rendered stable, both biomechanically and biochemically (Fig. 2G, H). Such stabilized fascia has found utility in urethral slings and shoulder rotator cuff repair.

Soft tissue—skin. Human allograft skin has been used for many decades as a temporary dressing to stabilize third-degree burns. This form of skin is the split-thickness tissue recovered with, and retaining at use, the epidermal layer. A newer form of skin is more thoroughly processed to remove the epidermis, resulting in a dermal layer that can be further treated to remove cells and achieve sterilization by chemical means (Fig. 2I, J). The preservation of natural channels facilitates cell and tissue in-growth and revascularization. Decellularized dermis is an excellent matrix for new tissue growth and has been successfully used in repair of abdominal ventral hernia.

Overall, bone and soft-tissue allografts described above have been very successful as surgical implants. They perform well because of their inherent biomechanical properties. However, the mechanisms of creeping substitution are only empirically understood. Allografts with their three-dimensional matrix framework can help in further studies of

cell-matrix interactions. For example, cancellous bone can be utilized as a matrix for studying osteoclast-osteoblast interaction to assist in elucidating the osteoporotic mechanisms. Skin grafts with primarily type I collagen, and articular cartilage grafts with primarily type II collagen could conceivably be experimental matrices to study the interaction of fibroblasts and chondrocytes, respectively. Further, the allograft matrices can be used to study the cell mechanisms underlying cell-matrix interaction. The details of the osteoclast-osteoblast mechanisms as a function of different matrices could be used to elucidate growth factor sequencing. Cortical, cancellous, long and flat bone, all may have significantly different forming mechanisms.

Arthur Gertzman concluded that human allografts represent a precious biomaterial made available by the generous gift of tissue by a donor family immediately after the unexpected death of a family member. These families are asked to donate tissue and, if they so choose, to designate the tissues for use in research. Human allograft tissue is available from MTF in limited quantities at no cost to accredited academic institutions and hospitals with research interests. The US tissue-banking industry is largely not-for-profit and has as part of its mission the support of research to honor the requests made by the donors and their families.

FUNCTIONAL TISSUE ENGINEERING OF LIGAMENTS AND TENDONS

Savio Woo from the University of Pittsburgh, Pittsburgh, PA, discussed the functional tissue engineering (FTE) of ligaments and tendons, with reference to the structural and mechanical requirements derived from healing.

Ligaments and tendons are bands of dense connective tissue that stabilize joint movement by transmitting forces. The biochemical and biomechanical properties of ligaments and tendons make them well suited to function in the unique environment of each particular joint. Unfortunately, both the medical collateral ligament (MCL) and the ACL of the knee joint are frequently injured during sports and work-related activities, with 100,000 to 200,000 tears occurring in the United States each year.[51] The MCL ruptures can heal spontaneously, while midsubstance tears of the ACL do not heal. Thus, to improve knee stability and allow patients an earlier return to preoperative levels of activities, surgical reconstructions using BPTB or hamstring tendons as autografts to replace the ruptured ACL are performed.[52,53]

However, there are issues related to the autograft harvest. Defects in the PT are not completely healed for months.[54–56] Lessening the severity of these complications by enhancing healing of the PT should lead to improved patient outcome.[57–60] The MCL, on the other hand, can heal but with a significantly different biochemical composition and matrix organization, resulting in inferior biomechanical properties even 2 years after injury.[61–64] Changes in collagen content, cross-links, and organization have been identified as three of the major contributors to the inferior biomechanical properties of the healing MCL. Therefore, efforts involving the use of FTE technology to enhance the healing of the ligament and tendons are of great interest.

Structure and composition of ligaments and tendons

Fibroblasts of ligaments and tendons are embedded within densely packed ECM.[65] The ECM is predominately a network of fibrillar collagen structures arranged in parallel along the long axis of the tissue. Between 65% and 70% of the total weight of ligaments and tendons is water. Type I collagen is the major matrix component (70-80% dry weight) and is primarily responsible for the tensile strength of the tissue.[66–70] Type III and V collagen make up to 8% and 12% of the dry weight, respectively.[70–72] To determine the biomechanical properties of ligaments and tendons, uniaxial tensile testing of bone-ligament-bone and bone/muscle-tendon-bone complexes is usually performed because the *in vivo* function of these tissues is to transmit tensile loads. The human MCL has an elastic modulus of 332.2 ± 58.3 MPa and a tensile strength of 38.6 ± 4.8 MPa.[73] Those for the central PT are 305 to 660 MPa and 57 to 64 MPa, respectively.[74–76]

The healing process

The healing of ligaments and tendons can be described as a four-phase process: Phase I is bleeding into the wound gap, Phase II is the inflammatory response and granulation of the tissue, Phase III is fibroblast proliferation and blood clotting, and Phase IV is clot replacement by disorganized collagenous tissue, which remodels with time (months to years). In the early healing phase, the collagen content, especially types III and V collagen, increased.[70] After weeks of healing, the total collagen content and hydroxypyridinium cross-link density became lower than that of control ligaments.[77] The collagen fiber diameter of the healing MCL at 60–70 nm is small when compared to the bimodal distribution of the normal MCL.[62,78,79]

The mechanical properties of the healing ligament's midsubstance remained substantially inferior to those of the intact ligament up to 1 year after injury.[80,81] To gain function, the cross-sectional area of the healing MCL continues to increase so as to compensate for the lack of improvement in the mechanical properties. Similarly, the healing tissue in the PT, after the central third is harvested for ACL repair, is also abnormal, with disorganized collagen alignment, altered ECM composition, and inferior tensile properties compared to normal PT tissue.[82–84] Further, the remaining PT becomes abnormal due to hypertrophy to a tissue of lesser quality (inferior mechanical properties).[82,84,85]

Novel treatment strategies based on FTE have shown some promise in restoring the normal function of injured ligaments and tendons.[86–91] These approaches include innovative biological and bioengineering techniques using

growth factors, gene transfer therapy, cell therapy, scaffolding materials, and mechanical stimuli.[92–94]

Cell function and matrix synthesis. In vitro, growth factors increased cell proliferation, cell migration, as well as ECM synthesis and production. In particular, fibroblast growth factor, epidermal growth factor, platelet-derived growth factor-BB, and transforming growth factors (TGF-β1 and TGF-β2) are effective.[95–99] However, when extending the *in vitro* findings to *in vivo* experiments, many contradictory results were found, suggesting that *in vivo* conditions are much more complex. Further investigations are needed to determine optimal dosage, carrier vehicles, and timing of applications for these exogenous growth factors.

Gene therapy has been used to mediate the production of specific matrix proteins, directly or indirectly. *Ex vivo* and *in vivo* gene transfer are often performed, in which viral vectors or liposomes can be used as carriers for genes.[100] In an *ex vivo* approach, a marker gene (LacZ) was successfully introduced and expressed in the rabbit MCL and ACL using an adenovirus as a vector.[101] To date, there have been various successes in the delivery of therapeutic genes to the PT.[100,102] Another technique, antisense gene therapy, is the delivery of genetic material that binds to signaling RNA and reduces the expression of an undesired gene. Currently, liposomes are also being used in our research center to deliver antisense oligonucleotides that reduce the expression of collagen types III and V, which, as discussed earlier, are elevated during the early stages of ligament healing.[103]

Cell and scaffold based approaches. Cell therapy is another potential method to enhance ligament and tendon healing. For example, bone marrow derived cells (BMDCs) have the potential to improve the healing process of large defects in the Achilles' tendon and PT.[82,88] The BMDCs have been shown to play an important role in wound healing[104–106] and can be obtained in high numbers with relative ease.[82,107] Recently, new methods geared toward PT healing have tried to fill the central third PT defect with collagen gels of different BMDC seeding densities. Improved mechanical properties were seen when compared to nontreated defects.[82,107,108] This particular cell therapy is attractive because the use of autogenous cells would minimize the immune response at the injury site.

The use of biological scaffolds, such as the porcine small intestine submucosa (SIS), offers distinct promise in accelerating ligament and tendon healing and regeneration.[108–112] The SIS possesses a structural hierarchy that is naturally arranged, and it is mostly composed of collagen type I. Forty percent of the SIS degrades within 1 month *in* vivo,[113] and its by-products have been shown to be chemoattractants for cells (including BMDCs).[114–116] Moreover, it contains many bioactive agents (growth factors, fibronectin, and so on)[117–119] and causes a limited inflammatory reaction.[120]

The potential of SIS to guide and support soft-tissue regeneration, promote ECM organization, and eventually improve the quality of the healing tissue has been demonstrated in the rabbit MCL model.[91,111] A single layer of SIS applied to a 6 mm gap injury improved healing at 12 and 26 weeks. At 12 weeks, the collagen content, as represented by hydroxyproline, in SIS-treated groups was 36% higher than that in the nontreated group. Moreover, the collagen type V/type I ratio, measured by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), was reduced to near normal levels at 12 weeks. Correspondingly, transmission electron microscopy demonstrated that larger diameter collagen fibrils started to appear with increasing numbers at 26 weeks due to SIS treatment. The SIS also improved mechanical properties of the treated MCL as compared to nontreated MCL: the tangent modulus and the tensile strength were 33% and 50% higher, respectively, at 26 weeks postinjury ($p < 0.05$).

Chemoattractant degradation products and bioactive agents of SIS could enhance healing.[114] The accelerated healing of the defect in response to SIS treatment allows better maintenance of stress- and motion-dependent homeostasis. Also, the SIS can be modified *in vitro* by seeding BMDCs on the scaffold and applying cyclic stretching in order to increase the alignment of cells, as well as improve the production and orientation of collagen. Hence, when applied *in vivo*, the scaffold could accelerate the initiation of the healing process, ultimately helping to make a better neoligament or tendon.

Mechanical stimuli. Different types of mechanical stimuli have been attempted to improve the reparative process of ligaments and tendons.[121–126] Cyclic stretching of cells from ligaments and tendons *in vitro* has been shown to cause increase in collagen synthesis[125,127] and changes in intracellular processes (i.e., different regulation of metabolic and inflammatory genes and calcium signaling).[126,128–132] Interestingly, fibroblasts align themselves via contact guidance from a substrate with microgrooves and along the direction of the maximum principal strain in collagen gels.[122] Similarly, *in vitro* studies have shown that multidimensional mechanical strains applied to BMDCs embedded in a collagen gel upregulated the gene expression of collagen types I and III and tenascin-C, which are typically expressed in fibroblasts.[123] When a bioscaffold is applied to a healing ligament or tendon *in vivo*, it will likely serve as a substrate that will provide contact guidance for cells. As a result, the healing neotissue will have more aligned collagen fibers with a concomitant improvement in mechanical and viscoelastic properties when compared to nontreated controls.

Savio Woo summarized that biotechnology has seen many recent exciting developments such as the sequencing of the human genome, stem cell based therapies, and the promise of tissue engineering. Still, however, these opportunities also present many challenges as the knowledge gained about a particular gene, protein, or cell is eventually translated to a clinical application. Solving such complicated issues will require that experts from different disciplines

work together in unison. The role of biomedical engineers within this framework can help link interactions at various levels of scale: molecules to cells, cells to tissues, tissues to organs, and organs to body function. Such a seamless interaction of talented biologists, biomedical engineers, and clinicians, as well as professionals from many other disciplines, will lead to therapies that allow injured ligaments and tendons to heal closer to that of normal. With the help of funding agencies aware of the need for this research, a team-based approach, and the new developments in FTE, the future appears to be very bright.

ISSUES IN THE TRANSLATION OF RESEARCH INTO PRODUCTS

Anthony Ratcliffe from Synthasome, a company based in San Diego, CA, discussed the challenges related to the conversion of the results of research into products that are profitable and meet regulatory requirements. Tissue engineering offers great promise for future products that have the potential to address unmet clinical needs and to substantially improve patient treatment. The technical areas to be concentrated on will be repair and replacement therapies, while technologies using rapid screening of new therapeutic agents may also have an impact. The conversion of interesting technologies and experimental results into products that meet regulatory requirements are challenging. Perhaps the greatest challenge is in the commercialization of the product. If a tissue engineered technology and concept are to be successful in the long term, there is no doubt that the clinical value must be accompanied by financial profit.

Tissue engineered products

The initial thrust of tissue engineering was driven by the envisioned commercial opportunities, and this has seen development of some new products, particularly in wound care and orthopedics. In wound care, TransCyte®, a dermal fibroblast-derived ECM on a sheet biomaterial, was introduced in 1997, for the treatment of third- and second-degree burns. Apligraf® and Dermagraft®, introduced in 1999 and 2001, respectively, contain allogeneic live cells and human ECMand are used in the treatment of chronic wounds such as venous and diabetic foot ulcers. In orthopedics, Carticel® (autologous cultured chondrocytes) was introduced in 1997 for the treatment of focal articular defects. In 2004, Infuse® was approved for spinal fusion, and consisted of the growth factor bone morphogenic protein 2 (BMP-2) within a collagen sponge (to regulate growth factor release) placed within a metal cage.

A commonality of these products is that they have been expensive to develop and produce, and once introduced to the market require substantial commercial commitment by their manufacturing companies to allow the products to become established within the clinical marketplace. All the tissue engineered products other than Infuse have struggled to approach profitability, in spite of their demonstrated clinical value to certain patient populations. Infuse now has robust sales and demonstrates that profitability in the fields of regenerative medicine and tissue engineering is possible.

Level of difficulty in developing new products

The technical challenges faced in the development of a new tissue engineered product must be of prime consideration, given that the field is complex even in its simplest forms. The more difficult the clinical challenge, the more technically complex the product is likely to be, and more difficult and expensive will be the product development pathway. It is useful to consider the mechanisms of tissue engineered products to be in one of the three general categories: (a) facilitate repair, (b) induce repair, and (c) replacement. The simplest mechanism by which a tissue engineered product can function is by facilitating repair, where the product participates in a passive manner to enhance the ongoing repair process. An example of this would be TransCyte, where the human ECM appears to enhance an ongoing repair process.

A more difficult mechanism for products is when they must induce a repair process. The Dermagraft and Apligraf products achieve this when they are placed into a chronic wound site where the normal repair activities are stalled, no longer able to move a repair process forward. These products, with live cells secreting a variety of growth factors that can participate in the wound-repair cascade, induce the wound-repair process to reinitiate. Carticel and Infuse also fall into this general category. The most difficult mechanism by which tissue engineered products can function is in the replacement of tissues or organs, where functionality of the implanted product is required at or near the time of implantation. There are no products on the market that fit this category, but envisioned products include articular cartilage that repairs large articular areas, blood vessel replacements, heart valves, and others.

As can be seen, the more difficult the functional task required by the tissue engineered product, the more technically challenging will be the product development. Increased technical challenges will be associated with increased development time cost, and probably increased risk of the product being successful clinically. The development of new products should therefore consider the level of difficulty when determining whether or not to proceed with a product development program. Technically simple products are likely to be introduced sooner. The more technically difficult products, where the clinical need is likely to be highest, will require more costly development pathways. However, it is in the interests of the health care community that work be done also on the development of these products since their development pathway is almost certain to be substantially longer.

Technical challenges. The product development pathway should be initiated with an assessment of what is necessary

in a product, its manufacture, and clinical performance to achieve the clinical objective. This process, sometimes referred to as ''Quality Functional Design,'' can prove to be invaluable in determining the vital characteristics that the product should have, and distinguishing these from the features that are less important. Three categories that may be considered are (i) what must you have, (ii) what should you have, and (iii) what would you like to have. This process should emphasize moving as much as possible out of the ''must'' category. If this is done effectively, then the product to be developed will efficiently address the clinical and manufacturing needs.

The work done so far in tissue engineering has clearly demonstrated how products might be developed, and what their limitations and challenges are. There are many key technical hurdles in the field of tissue engineering, and in the development of any new tissue engineered products, and one or more of these are likely to be hurdles that must be overcome. These include

- Cell source
- Mechanical properties
- Vascularization
- Immune response
- Biocompatibility
- Scale-up for manufacture
- Storage/shelf life

New technical advances and innovative manufacturing may be necessary to allow product manufacture to meet the envisioned clinical needs and be cost effective.

The development of industry standards will enhance the efficiency of product development and regulation. The standards organizations, including ASTM and ISO, are in the process of developing these standards, and when they are established using consensus and with input from regulatory authorities, they can enhance the product development pathway significantly.

Regulatory pathways, associated costs, and timelines. Tissue engineered products must be regulated to ensure safety and effectiveness. In the United States, the FDA will most probably regulate a tissue engineered product either as a device, biologic, or drug. The quickest and least costly regulatory pathway is as a device with a 510(k) designation. Outside of this, once products have undergone development and are ready for their key preclinical animal studies, it is likely to take 5 years and $30–$200 million as a device, and 8 years and $50–$300 million as a biologic or drug.

While product development time may be shorter and costs less, it is most likely that the time will be longer and the costs higher. These timelines and approximate costs are key to considering if and how to develop a concept into a product. The technical risks and predicted financial returns are in a continual balance, and the higher predicted financial returns can motivate taking higher technical risks. However, it is important that realistic financial predictions are made, otherwise profitability will not be reached and the product will not have longevity.

Potential market sizes for tissue engineered products

The general markets that tissue engineered products are likely to be placed are usually extremely large, multibillion-dollar markets. However, realistic assessment of the true market size for tissue engineered products usually shows that the products will be used in niche areas where the total revenue is predicted to be approximately $20–$200 million per year. These are modest revenues, and they emphasize the importance of realistic business assessments. When these levels of revenue are compared to the potentially long development timelines and associated costs, the return on investment is sometimes not particularly convincing. This in turn argues for minimizing technical challenges that new product development must address. While the financial returns for the current products, and for most of the envisioned products, are not high, it is likely that profitability can be reached when a robust business plan is developed and followed.

Key administrative hurdles for tissue engineered products

The administrative hurdles, like the technical hurdles, are substantial, and include the regulatory system, the intellectual property rights, and funding of product development through to profitability. The regulatory pathways in the United States and other major markets worldwide require a substantial body of evidence that shows safety, effectiveness, and efficacy of the product. The manufacturing of a product must undergo strict and thorough development, validation, and monitoring. This rigorous regulation of products is critical for the industry. Limitations include the uncertainty in regulatory requirements for each individual product and the nonuniformity of regulatory systems between the major international regulatory agencies.

Patent protection for an individual product is a critical feature of product development. Developing new patents is costly, the outcome is uncertain, and it must occur near the beginning of product development. The limited time for patent protection (usually 20 years from initial submission) requires that the product development pathway be followed in an efficient manner; otherwise patent protection will be lost by the time profitability arrives.

The funding of new product development, particularly at the early, high-risk stages, is always going to be difficult. This problem has increased recently, with the usual venture capital funding being concentrated on the late-stage product development, where risks are lower and timelines are shorter. The federally funded Small Business Innovation Research programs have been successful at initiating product development; however, the funding of midstage product development has become a major limiting factor in tissue engineering. This funding gap represents a major administrative hurdle

that must be planned for using a robust business model. Otherwise, the early research and development efforts will be wasted.

Recommendations for product development

The development of engineered products should incorporate the following in the product development pathway:

1. *Maximize the knowledge base and infrastructure.* This requires that academic and industry groups form collaborative teams working for the same coordinated goals. The academic groups can provide detailed technical knowledge and input; the industry groups can more readily assess product development issues. A functional team approach will maximize progress.
2. *Use good science and engineering.* The early phases of any new technology require risk taking and leaps in technical understanding, rather than incremental advances. However, whatever stage the technology is in, it should be based on high-quality science and engineering. Without this, the product development efforts will not be robust, and will be prone to failure, at early, middle, or late stages.
3. *Involve, listen, and respond to regulatory agencies.* A collaborative approach with the regulatory agencies will lead to good product development. It is unfortunate that these agencies are limited in resources, and new technologies such as tissue engineering present difficulties that they must address for safety. However, their input must be maximized. Ultimately, their requirements must be met for a product to get to market.
4. *Have robust and realistic business plans.* Generate revenue early, create value, and minimize the need for major and long-term investment. Avoid overly optimistic market projections, anticipate hurdles, and develop contingency plans. Technical success but commercial failure does not generate a product. The best interests of the R&D, investors, and patients will all be served by using a realistic business plan.

Anthony Ratcliffe summarized that the technology of tissue engineering has been shown to be feasible, products are already on the market, and there is the potential for new products to be developed that have significant clinical impact. The technical and administrative hurdles are substantial, but with realistic technical, regulatory, and business planning, success should be assured.

THE PUTATIVE NEED FOR INCREASED BASIC RESEARCH IN THE FIELD OF TISSUE ENGINEERING

Michael J. Lysaght from Brown University, Providence, RI, challenged the widely floated proposition that commercial and clinical failures of early tissue engineering products demonstrate a need for more focus on basic research, and a clearer understanding of interactions between living cells and biomaterials. He argued that such a perspective is largely *a priori* and based upon the misconception that the starting difficulties of tissue engineering represented scientific failures. In fact, early trials and tribulations in this field have been the result of underpowered clinical trials, flawed business plans, ineffective marketing, and a Procrustean regulatory stance by the FDA. Basic research and scientific understanding are eminently worthwhile in their own right, but they cannot, and will not, in the perspective elaborated here, contribute much to the realization of tissue engineering and regenerative medicine.

The early experiences of tissue engineering represent a unique, and still unfinished, chapter in the history of contemporary biotechnology. The field had its beginnings in the seventies with early research into artificial skin and the biohybrid pancreas, two initial therapeutic constructs that combined biomaterials and living cells. Isolated investigations continued throughout the eighties, but the field acquired its own identity and significant momentum during the nineties. Commercial failure of early tissue engineering products at the turn of the century and the demise of the flagship tissue engineering firms led to turbulent upheaval, restructuring, and reassessment. The implicit leitmotif of the NIH workshop Tissue Engineering—The Next Generation was that more and better fundamental science is needed to rescue tissue engineering from a premature demise. The leading thinkers in the field convened to outline the intellectual pathways (aka roadmaps) describing the structure of this new scientific knowledge. Dr. Lysaght offered a contrary viewpoint to this premise.

The growth of tissue engineering and its macroeconomic structure are well documented. During the period from 1990 to 2003 (latest available data), the private sector invested nearly $4.5 billion in the field. Most of the money went into small start-up firms with anywhere from a handful of employees to a workforce numbering in the several hundreds. At the end of 2000, the tissue engineering industry boasted 73 companies with over 3,000 employees. The capital value of the six largest public companies, none of which yet had significant products, was $2.5 billion. Even by its most generous estimates, government and foundation support constituted less than 10% of the total funding. Smaller firms spent their money on product development; larger organizations on activities related to regulatory approval. The goal was rapid progression to the clinic, and there was very little in the way of resources and enthusiasm for basic research or fundamental inquiry. Lack of federal support was considered unfortunate but was not regarded as limiting. Industry worked closely with academia and supported work at university, but very pragmatic focus attended even these collaborations.

Starting in 2001, things began to go wrong for the field. In short succession, Organogenesis and Advanced Tissue

Sciences launched their "Living Skin Equivalents," the market reception and sales of which were sufficiently disappointing to drive both companies to bankruptcy; over 500 workers in the field became redundant. CytoTherapeutics and Circe completed phase III clinical trials of their products without the statistically sufficient efficacy data needed to win FDA approval. Diacrin and Vitagen abandoned clinical trials in phase II because of, respectively, clinical difficulties and financial constraints. These four companies directly or indirectly ceased operations, leading to a further loss of about 400 positions. And then came the stock market crash, which reduced the capital value of publicly traded tissue engineering companies by 90%. The investing public lost its appetite for these high-risk ventures, forcing the remaining companies to downsize their workforce and stretch out their business plans. About half of all employees in tissue engineering found their jobs eliminated. Very fortunately, stem cells had now entered the picture and much of the dislocated workforce, sadder but wiser, was easily assimilated.

The field has changed rapidly since 2003. Venture capital plays a much smaller role. Large firms, for example, Genzyme, Medtronics, and J&J, have begun devoting serious resources to focused projects. Low-risk products, for example, textured matrices to foster wound healing but which contain no living cells, are increasingly attractive. Interest in stem cells, which may or may not be related to tissue engineering, has blossomed. At this time (October 2005) only one product meeting the classical definition of tissue engineering is in FDA efficacy trials. Tissue engineering still seems destined to play a major role in 21st-century medicine, but its emergence is likely to take longer than originally envisioned.

Meanwhile, the academic community has responded with relish to the challenge of defining what went wrong with tissue engineering first time around, and to reappraising the strategy for advancing this technology in the future. This is certainly understandable. Academia played a subordinate rather than a leading role in tissue engineering in the nineties. And the fact is that the "waste" of 5 billion dollars in the first generation of tissue engineering—in the sense that no successful clinical product has emerged—is certainly grating within a community where success or failure is measured in terms of research grants usually valued at $250,000 per investigator per year. The prescription, certainly explicit in other contributions to this workshop, is clear: more basic research, more understanding of the interactions between living cells and synthetic materials, more and better biomaterials, better cell biology, and the like. All such pursuits are worthwhile, even laudable, and all are worthy of support in their own rights. But, by themselves, none would have been likely to either change the fate of the first generation of tissue engineering or alter the course of future efforts. Rather, the need is for improved product development, better management and sounder business plans, and a somewhat more coherent regulatory environment.

The view that more basic research would enable the clinical realization of tissue engineering is generally floated as an *a priori* assertion. Proponents do not provide an inventory of missing knowledge and then elaborate how its availability would have impacted the fate of Dermagraft or Circe's bioartificial liver. Such an explanation would be hard to support simply because the failure of these products was far more grounded in the business side than in the scientific side. And until such a reckoning is made, the call for more basic research represents more of a faith-based initiative than one grounded in solid evidence.

The history of first-generation artificial organs (hemodialyzers, heart valves, pacemakers, etc.) is instructive. These devices first demonstrated the possibility of replacing deteriorated natural organs with man-made substitutes and arguably breached a more significant development chasm than will be required for tissue engineering. But the emergence of first-generation devices was largely a matter of trial and error. Very little basic and clinical science was involved; engineering was primitive; and biomaterials science was highly Edisonian. Moreover, once organ replacement got past its start-up problems, which are not very different from those now being experienced by tissue engineered products, its further growth was fueled more by skill at medical management than by advances in basic science. For example, to this day, nephrologists are just not sure what a hemodialyzer has to remove.

Dr. Lysaght argued that, in any event, the real reason why more basic scientific research is not going to "fix" tissue engineering is that the field's start-up problems have mostly not been scientific but rather related to business and regulation. Consider living-skin equivalents, itself a telltale misnomer. These products failed, and led to the bankruptcy of the parent companies, for a variety of reasons none of which could have been remediated by a more extensive science base. First, the firm's sales forecasts were vastly inflated. Ramp-up times were hugely underestimated. In a classic case of technology push rather than market pull, the companies simply did not appreciate the difficulty in getting practicing dermatologists to change their treatment methods for slightly improved outcomes. They also failed to create a suitable reimbursement environment, one that provided a positive-sum proposition to the practicing dermatologist. The products were likely mispositioned as skin substitutes when they were really wound-healing agents. The basic calculus of the agreements between the producing firms and the marketing partners was sufficiently off-kilter to provide neither party with appropriate incentives. It is encouraging to note that most of these issues have been resolved and the sales of these products are currently both profitable and growing—without more basic research.

Several metabolic devices also failed to win FDA approval after pivotal or phase III clinical trials. This again was not due to a lack of basic science but rather due to a lack of funds to field trials large enough to reach statistical significance of benefit. Enrolment for efficacy trials of tissue engineered products was typically 1 to 300 patients; drug trials usually run in the several thousands. In the case of Circe's

bioartificial liver, a clearly responding and statistically significant benefit was identified for a subset of patients, but FDA approval was denied since this group had not been identified in advance.

Perhaps at the root of these problems is the mentality of the venture capital groups, who supplied much of capital for the industry and who maintained effective board-level control well into the period of greatest difficulty. The venture capitalists (VCs) implicitly imposed the business model that was proven lucrative and successful for earlier biotechnology start-ups based upon recombinant molecular biology. Firstly, a large number of failures could be tolerated because of the offsetting benefits of the occasional success. Values of a start-up increased in formulaic fashion with achievement of a number of specific milestones: clinical success in small animal models, publication in a high impact-factor journal, corporate partnership, first clinical exposure, and regulatory-level clinical trials. These milestones became ends in themselves; their achievement was crucial to firms raising the capital needed to subsist and, often, to providing an exit strategy for the VCs. Speed was of the essence, and a prevailing belief was that firms with much fewer than 150–250 employees were simply too constrained to succeed.

High-risk ''bet-the-farm'' strategies often took precedence over the more methodical development and iterative experimentation suitable for medical devices and implants. Firms had to target very large markets from the outset to justify the high cost of rapid development and thereby missed the opportunity to gain initial clinical exposure in cases where risk/benefit ratios would be more favorable. The cumulative effect was a tissue engineering industry with a Ponzi-like ethos, until things went south in 2001–2003 with the loss of about $2.5 billion in capital value in less than a year. The encouraging news is that these mistakes are unlikely to be repeated anytime soon; firms leading the recovery of tissue engineering are restrained in their enthusiasm for success. The relevant conclusion is that all the basic research in the world would not have let the industry avoid the inevitable consequences of its flawed business models.

Finally, there is the impact of regulation on the field. Tissue engineered products that were regulated as devices by the Center for Devices and Radiological Health fared reasonably well, those regulated as drugs (by the Center for Drug Evaluation and Research) or as biologics (by the Center for Biologics Evaluation and Research) did not, and, in fact, these bureaus have yet to approve a single tissue engineered product. The reason for this relates to the economics of devices and drugs approval. The cost of regulatory approval for a life-sustaining or life-supporting medical device averages to $50 million, and the corresponding cost for even a me-too drug is around $800 million. Drug reviewers are used to exhaustive procedures, large randomized double blind trials with populations in the thousands, and a ''cost-be-damned'' approach by applicants. The relatively modest efforts of tissue engineered products simply failed to meet these expectations. The FDA has a genuine desire to promote new medical technology, and considerable dialog has taken place between agency management and advocates for tissue engineering. But events such as the Vioxx episode tend to reinforce a culture of extreme caution.

Michael Lysaght summarized what tissue engineering needs to bring its promising concepts to clinical practice: long-term rather than short-term investment money, business plans geared to realistic cost/benefit trade-offs, less hype, more sophisticated regulatory staff, and engineers skilled at product development and manufacturing scale-up. Basic research will not help much. Is this an argument against support of basic research? Absolutely not. Such research has an almost unlimited capacity to advance the health and well-being of mankind. It should be supported as lavishly as societal resources permit, but for the right reasons.

TISSUE ENGINEERING PRODUCTS: SUCCEEDING OR FAILING DEPENDS ON INTERPRETATION OF THE DEFINITION

Arthur Coury from Genzyme Corporation, Cambridge, MA, presented a talk on tissue engineering initiatives at this organization, which was of great interest because of Genzyme's long-term efforts in the field, its set of cell-based products, and its commitment to developing advanced tissue engineering products. Genzyme is one of just a few corporations that have been able to maintain their commitment to cell therapies for well over a decade, ''toughing'' it through the difficult development process and unprofitable times until profitability was achieved. The Carticel autologous cartilage implant became profitable in 2005, and Epicel© autologous keratinocyte graft is maintained as a humanitarian product to save the lives of serious burn victims. Some would interpret definitions of tissue engineering in the narrow sense of applying cells; cells and scaffolds; or cells, scaffolds, and factors for therapeutic purposes and would conclude that tissue engineering has, with few exceptions, been a failure. Dr. Coury addressed tissue engineering in the context of proffered definitions, and how their interpretation can affect perceptions and policies relative to its status and advancement, using Genzyme initiatives as examples embodying the proposed scope of the field.

Scope of tissue engineering

Most published definitions of tissue engineering suggest a broad scope. For example, Gordana Vunjak-Novakovic and David Kaplan, for this conference, stated: ''Tissue Engineering has been defined as the application of the principles and methods of engineering and the life sciences towards the development of biological substitutes to restore, maintain, or improve functions.''[133] Langer and Vacanti's pioneering definition was very similar, defining tissue engineering as

"[a]n *interdisciplinary* field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve *tissue* function."[10] David Williams, in his *Dictionary of Biomaterials*, provides a detailed, yet still broad definition: "Tissue Engineering is the persuasion of the body to heal itself, through the delivery to the appropriate sites of molecular signals, cells and supporting structures."[134] In 2005, Dr. Coury suggested a broad definition, intentionally lacking in detail: "The generation, regeneration, augmentation or limitation of the structure and function of living tissues by the application of scientific and engineering principles."[135] Any of these definitions covers a broad range of therapeutic approaches leading, basically, to the control of cells and their matrices (Table 5).

Wound dressings and tissue sealants

The choice of a wound dressing (occlusive, hydrogel, or gauze) would have tissue engineering implications, as each dressing induces a different rate of healing and potential outcome. Many engineering and scientific principles, such as adherence, permeability, leaching, and lamination, are manifest in wound dressings. The dressings are intended to effect guided tissue regeneration, a component of tissue engineering as defined above. If wound dressings and related wound-care products are tissue engineering products, then this segment of the field is a very large, profitable, and multibillion-dollar one[136] (Table 6). Therapies such as spinal fusion using demineralized bone, skin expansion using expandable implants, or bone-lengthening using mechanical prostheses and surgical techniques would fit Dr. Coury's interpretation of tissue engineering.

Surgical sealants (Table 6) are not normally considered tissue engineering products. They are poly(ethylene oxide)-based, tissue adherent hydrogel coating, resorbable over 3–4 months, and formed from a liquid photopolymerizable macromer formulation applied to dural lesions (FocalSeal). In a rabbit study, a 4 mm^2 dural excision was made above the brain, and the opening was sealed with an experimental sealant, using a priming technique that only adhered the hydrogel to the dura, not the brain (Fig. 3A). After 10 days, the dural site was examined and found to have formed a contiguous, fibrovascular film under the hydrogel, which sealed the excision and would mature to a strong, autologous tissue seal (Fig. 3B). Without the sealant, the dural leak was sealed by the brain forming adhesions to the soft tissue above the brain (Fig. 3C). The effect produced by the sealant provides a persuasive example of guided tissue regeneration, and should thus be considered tissue engineering. Guided healing has been observed for hydrogels used as dermal wound dressings and adhesion prevention barriers, as well as surgical sealants. Examples could be given of cell and matrix control by tissue engineering products for all of the approaches listed in Table 6, advocating for an expanded perception of tissue engineering.

Replacement parts and electronic devices

Some would say that the broadened definition dilutes the impact of a focused concept, and sounds like it is all-inclusive. However, many therapies would not be included. Replacement parts such as prosthetic hips and knees, orthopedic plates and screws, dental implants, crowns and fillings, intraocular lens, vascular grafts, circulatory assist devices, and heart valves would be excluded. Implantable delivery devices such as central venous catheters, drug pumps, and percutaneous access devices do not meet the criteria. Free-standing diagnostic devices would be excluded.

Electronic devices such as cardiac pacemakers and neurological stimulators would fall into a gray area. They definitely affect the function of responsive cells, but they also replace the function of defective cells and matrices. To the extent that the function of electronic devices is preserved or enhanced by cell and matrix (e.g., fibrosis) control (e.g., drug-eluting pacemaker leads), tissue engineering is involved. Thus, some segments of this $10 billion market[137] could be categorized as tissue engineering. Drug-eluting coronary stents form another family of products (device-drug combination products) that can either be placed in the tissue engineering category or be excluded. On the con side,

Table 5. Tissue Engineering Therapeutic Approaches

- Guided Tissue Regeneration
- Organ Generation/Regeneration
- Tissue Reinforcement
- Tissue Augmentation/Space Filling
- Healing Modulation
  - Hypertrophy Inhibition
  - Fouling Prevention
  - Tissue Adhesion/Sealing
  - Adhesion Prevention
- Enhanced Mitosis/Matrix Deposition
- Organ Function Modulation
- Expression of Bio-Actives
- Gene Therapy
- Protein Therapy
- Etc.

Table 6. Wound Care Markets, 2003

Surgery and Trauma:	*$4.3 Billion*
Burns:	*$1.5 Billion*

Components:

- Sutures, Staples, Adhesives, Sealants
- Anti-Infective, Cleansing, Debridement Products
- Tapes, Dry Dressings
- Moist Dressings (Hydrogels, Hydrocolloids, etc.)
- Biologicals (Bio-Artificial Skin, Collagen, Growth Factors, Other)

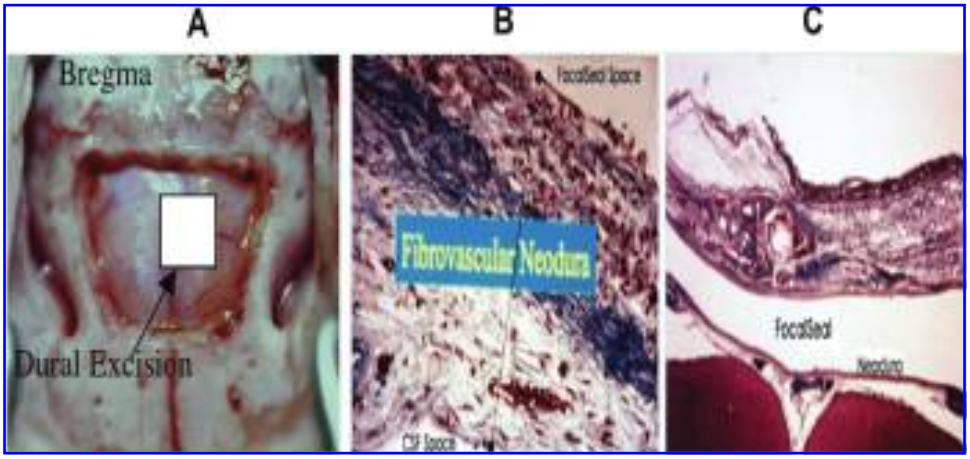

FIG. 3. Rabbit study used in the development of Genzyme's dural sealant product, a poly(ethylene oxide)-based, tissue adherent resorbable hydrogel. (**A**) Dural excision of 4 mm^2 was made above the brain, and the opening was sealed with an experimental sealant, using a priming technique that only adhered the hydrogel to the dura, not the brain. (**B**) After 10 days, the dural site was examined and found to have formed a contiguous, fibrovascular film under the hydrogel, which sealed the excision and would mature to a strong, autologous tissue seal. (**C**) Without the sealant, the dural leak was sealed by the brain adhesions to the soft tissue above the brain. Color images available online at www.liebertpub.com/ten.

their function is to replace the lumen of stenosed or occluded vessels. On the pro side, drug-eluting coronary stents, in particular, which dominate the $6 billion stent market,[138] function to control the cellular response and matrix production locally, and it could be argued with much justification that this is true tissue engineering.

Discussion and recommendations

Taken together, the exemptions from tissue engineering therapeutic products constitute a majority of the therapeutic device and combination device markets, which exceed $100 billion worldwide.[139] Dr. Coury estimated that a very respectable 20–30% of the total may be considered tissue engineering products, much higher than the few tens of millions of dollars attributed under limited interpretations.[140] Any discussion of pharmacologic therapy alone, which can, indeed, exert control over cells and matrices, is beyond the scope of this discussion.

Another argument against the expanded interpretation of tissue engineering is that the narrow concept is well established and entrenched, and it would be hard to reverse this perception. Dr. Coury argued that, in spite of the similarities of most definitions, there is no widespread agreement on the limits of tissue engineering. Some would argue that isolated cells are always involved. Disagreements could ensue if scaffolds were not included. Others would say that scaffolds alone could constitute the tissue engineering therapy if tissue regeneration occurs. Some, not all, would insist that the delivery of growth factors to enhance tissue generation along with a device is tissue engineering. The debate, even over the most likely candidates for tissue engineering therapeutics, attests to the lack of general agreement on the specifics of the concept. There is room for influencing the interpretation.

A final argument to be considered is based on the image of tissue engineering, which fails to achieve its promise in its projected time frame. Of some 20 plus companies listed by *Business Week* as tissue engineering start-ups in 1998,[141] only three exist today as intact companies, and they are not profitable from sales of tissue engineered products. Investment in narrowly interpreted tissue engineering in the United States has dropped substantially since peaking in the year 2000.[140] One side would believe that expanding the scope of tissue engineering to successful products would somehow reflect negatively on the latter in terms of public image and growth potential. The opposite effect could be the one to materialize. Successful products have made it because of medical needs being satisfied. The products are efficacious and demand is generally growing nicely, based on their effectiveness with a compound annual growth rate of 9%.[139] The reputation earned by successful therapeutic products over decades, included in the expanded concept of tissue engineering, should enhance the image of the field and bring many benefits. There is the public relations benefit of a successful image. Success and continuing optimistic growth projections attract public and private investment funding for research, development, and education. The interpretation that major successful therapies come under the umbrella of tissue engineering will attract students for careers in this field, and enhance their optimism for its robust growth.

The arguments for inclusiveness of therapies in tissue engineering to demonstrate successes are in no way meant to minimize the challenges to be faced in achieving the goals of regenerative therapy embodied in the narrower interpretation of the field. We are years away from off-the-shelf or even autologous cultured or internally generated commercial structures such as blood vessels, bladders, corneas, nerves, and menisci, even though clinical prototypes exist.

We are decades away from more complex structures (kidney, liver, heart, pancreas tissues).[142] But most of the principles required to achieve even the loftiest of our tissue engineering goals are known and applicable. Utilization of these principles with resources, persistence, and dedication will eventually but inevitably lead to the envisioned tissue regeneration. But this will require an environment that promotes the necessary research and development in an atmosphere of optimism and commitment.

Genzyme took the visionary position of executing the long-term development of its cartilage regeneration product using retained earnings from other products and supported it during its slow adoption. The company's commitment extends to the development of improved cartilage regeneration therapies, surgical techniques, and multiple uses for its cell therapies, as well as to other devices such as adhesion prevention products that are included in the broad tissue engineering interpretation. Its position for further exploiting tissue engineering therapy is excellent. Other formulas for tissue engineering product development, whether through venture capital, public funding, or private investment, will require vision, optimism, and persistence.

Arthur Coury concluded that the environment for generating the necessary support to advance tissue engineering would be promoted by the expanded interpretation of the definition, because it highlights great successes and promotes optimism, which could lead to increased funding and human resource commitment to the field. We are on stable ground here—there is robust justification that many more therapeutic products really do exert control over cells and matrices via scientific and engineering designs than are commonly considered.

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